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Design, synthesis and molecular docking study of new purine derivatives as Aurora kinase inhibitors

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ABSTRACT

Nine new purine-based compounds were designed and have been synthesized through series reactions of the starting compound 8-amino-substituted purine **(2)** with various reagents. Full characterizations of the synthesized compounds were performed to elucidate their chemical structures by means of physical and spectroscopic methods. All products were *in-vitro* tested for their potent anti-cancer action against different human cancers; leukemia (HL60), lung cancer (A549), Breast cancer (SKBR3) and Stomach cancer (MKN45). Upon experimental biological evaluation, synthons showed higher efficiency against SKBR3 cell lines in particular and hence were docked with anticancer enzyme (Aurora kinase) to adequately understand the link of each entity inside the enzyme active site.

1. Introduction

Among the numerous important heterocyclic compounds, purines are the most widely distributed kind of nitrogen-containing moieties present in nature [1]. They are consisting of fused pyrimidine to imidazole rings and found in important bi-molecules, such as adenosine triphosphate (ATP), guanosine 5′-triphosphate (GTP), cyclic adenosine monophosphate (AMP), nicotinamide adenine dinucleotide hydrogen (NADH), and coenzyme A [2]. They provide essential hydrogen bonding capabilities that make them interesting scaffolds to target a wide range of biosynthetic, regulatory and signal transduction proteins (e.g. G proteins, cell phosphates and polymerases) [3]. Purine derivatives constitute an extensive class of compounds (e.g. methylxanthine, caffeine, and theophylline), which are well-known for their therapeutic utilities as analeptics, antiasthmatics, vasodilators, antihypertensive, diuretics, anti-HIV-1, antimicrobial, bronchodilators, and anticancer agents [4-6]. Traube synthesis was reported to prepare most of purines by fusing pyrimidine diamines to imidazoles or by binding the pyrimidine ring to imidazole moieties [7]. Serine/threonine kinase mammalian Aurores (Aurora-A, -B, and -C) are the most prevalent mitotic progression regulators and are often abundantly expressed in human cancers at measured levels [8-12]. Latest studies demonstrated their role in promoting progression of the tumor, through the activation of reprogramming epithelial-mesenchymal transformation to lead to tumor-initiating cells' genesis [13] and inhibiting the growth of fulvestrant resistant T47D breast cancer cell lines as well [14]. Recent evidence suggests that the Aurora A and B (the two major types of Aurora kinase), tend to play a key role in the regulation of chromatid divergence [9,10]. More specifically, it allows the splitting cell to provide its daughter cell with its genetic material [15-18]. Inadequacies genetic instability, strongly linked to tumorigenesis can occur in this segregation [19]. Thus, the development of Aurora oncogenic inhibitors may boost cancer patients' clinical outcomes. In modern drug design process, molecular docking is very important as an optimization tool to illustrate the "best-fit" ligand orientation in binding to a specific protein and to deduce the assembly of the intermolecular complex formed with minimum binding energy for predicting affinity and activity [20]. The combination of two or more pharmacophores in a single molecule is one of the major approaches for designing of new biologically active moiety. Structural fragments with heterocyclic moieties are most obvious among all drugs [21]. Based on the above data and our efforts to develop new potent molecular anti-tumor agents [22-27], it is worth reporting the synthesis of new derivatives incorporating purine-imidazole core structure and *in vitro* investigation against various human cancers: leukemia (HL60), lung cancer (A549), Breast cancer (SKBR3) and Stomach cancer (MKN45). The compounds were also examined for their cytotoxicity against Lung normal cell lines (WI38) in order to indicate their toxicity. The structural activity relationship (SAR) study for the synthesized hybrids regarding antitumor activities was discussed. On the other hand, Reversine is known as a potent inhibitor of the mitotic kinase Mps1 [28] and has the potential to induce selectively cell death of cancer cells

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[29]. Therefore, a molecular docking analysis was performed on the synthesized compounds compared to Reversine to explore a creative class of Aurora inhibitors based on purine scaffold and realize the binding modus of each molecule in the Aurora enzyme active site.

2. Experimental

2.1. Chemicals and instrumentations

Chemicals and instrumentations involved in this research are fully identified and described elsewhere [30] and in supplementary file (S1).

2.2. Biological Analysis

The cultured cancer cells (DS Pharma Biomedical Co., Ltd., Osaka, Japan), cytotoxicity assay method and statistical analysis were previously reported [30] and fully described in supplementary file (S1). The relative cell viability in percentage was calculated according to the equation (1).

$$\%\ Cell\ viability = \frac{Abs_{570}\ treated\ sample}{Abs_{570}\ untreated\ sample} \times 100. \quad (1)$$

2.3. Docking methodology

The molecular docking technique was performed by using MOE 2015.10 software (The Chemical Computing Group Inc., Montreal, Canada) [31].

2.4. Synthesis

2.4.1. 8-Amino-1,3-dimethyl-3,4,5,7-tetrahydro-1H-purine-2,6-dione (2)

A mixture of 8-chloro-1,3-dimethyl-3,7-dihydro-1H-purine-2,6-dione (1; 0.43 g, 20 mmol) and excess amount of concentrated ammonia solution (20 mL) in ethanol (20 mL) was heated at 100 °C in a sealed tube for 2 h. The reaction mixture was allowed to cool at room temperature and left overnight. The collected product (2) was washed with cold spirit several times and recrystallized from ethanol to yield colorless powder (2.4 g, 61 %). M.p. > 310 °C (Lit. > 310 °C) [32,33].

2.4.2. General method for preparation of 8-substituted diazenyl-1,3-dimethyl-3,7-dihydro-1H-purine-2,6-dione derivatives (8–11)

A soln. of 8-amino-1,3-dimethyl-3,4,5,7-tetrahydro-1*H*-purine-2,6-dione **2** (3.90 g, 20 mmol) in 5% HCl soln. (20 mL) was cooled to 0–5 °C, and then $NaNO_2$ (1.38 g, 20 mmol) was added in four portions. The mixture was slow stirred for 1.5 h for complete diazotization. Coupler components **(4–7)** (1.88 g, 2.88 g, 2.88 g and 4.10 g, respectively, 20 mmol) were added in small portions with continuous slow stirring at cold condition for further 1 h. The yielded precipitates **(4–7)** were filtered off, washed with cold water, dried and recrystallized from ethanol.

2.4.2.1. 8-((4-Hydroxyphenyl)diazenyl)-1,3-dimethyl-3,7-dihydro-1H-purine-2,6-dione (8). Brownish red crystals (3.4 g, 57 %). M.p. > 300°C. IR: ν_{max}= 3165 (NH), 1687 (CO), 1651 (CO), 1550 (C=N) and 1418 (CH_3) cm^{-1}. ^{1}H NMR: δ/ppm= 3.20 (s, 3H, C<u>H</u>$_3$–N1), 3.41 (s, 3H, C<u>H</u>$_3$–N3), 6.79–7.91 (m, 4H, Ar–<u>H</u>), 9.98 (s, 1H, O<u>H</u>), 11.52 (s, 1H, N<u>H</u>–N7). ^{13}C NMR: δ/ppm= 28.39 (<u>C</u>–N1), 30.21 (<u>C</u>–N3 purine), 116.14, 116.87, 127.09, 128.70 (4<u>C</u>–phenyl), 119.24 (<u>C</u>–N–phenyl), 126.27 (<u>C</u>5–purine), 146.04 (<u>C</u>8–purine), 150.91 (<u>C</u>6–purine), 151.68 (<u>C</u>2–purine), 154.49 (<u>C</u>4–purine), 159.95 (<u>C</u>–OH). MS (*m/z*, %): 300 (M^+, 25.4). HRMS calc. for $C_{13}H_{12}N_6O_3$: 300.0971, Found: 300.0987.

2.4.2.2. 8-((2-Hydroxynaphthalen-1-yl)diazenyl)-1,3-dimethyl-3,7-dihydro-1H-purine-2,6-dione (9). Scarlet red crystals (3.7 g, 53 %). M.p. > 300°C. IR: ν_{max}= 3341 (OH), 3059 (NH), 1688 (CO), 1649 (CO), 1540 (C=N) and 1406 (CH_3) cm^{-1}. ^{1}H NMR: δ/ppm= 3.29 (s, 3H, C<u>H</u>$_3$–N1), 3.40 (s, 3H, C<u>H</u>$_3$–N3), 6.44–7.95 (m, 6H, Ar–<u>H</u>), 8.85 (s, 1H, O<u>H</u>), 11.28 (s, 1H, N<u>H</u>–N7). ^{13}C NMR: δ/ppm= 28.39 (<u>C</u>–N1 purine), 30.21 (<u>C</u>–N3 purine), 123.15, 123.45, 126.32, 126.51, 127.09, 128.70, 129.34, 140.17, (8<u>C</u>–naphthyl), 126.27 (<u>C</u>5–purine), 135.98 (<u>C</u>–N–naphthyl), 146.04 (<u>C</u>8–purine), 150.90 (<u>C</u>6–purine), 151.66 (<u>C</u>2–purine), 154.48 (<u>C</u>4–purine), 159.95 (<u>C</u>–OH–naphthyl). MS (*m/z*, %): 350 (M^+, 100). HRMS calc. for $C_{17}H_{14}N_6O_3$: 350.1127, Found: 350.1181.

2.4.2.3. 8-((1-Hydroxynaphthalen-2-yl)diazenyl)-1,3-dimethyl-3,7-dihydro-1H-purine-2,6-dione (10). Reddish brick crystals (4.6 g, 66 %), M.p. > 300°C. IR: ν_{max}= 3339 (OH), 3141 (NH), 1684 (CO), 1638 (CO), 1574 (C=N) and 1409 (CH_3) cm^{-1}. ^{1}H NMR: δ/ppm= 3.29 (s, 3H, C<u>H</u>$_3$–N1), 3.44 (s, 3H, C<u>H</u>$_3$–N3), 6.42–8.10 (m, 6H, Ar–<u>H</u>), 8.99 (s, 1H, O<u>H</u>), 11.29 (s, 1H, N<u>H</u>–N7). ^{13}C NMR: δ/ppm= 28.22 (<u>C</u>–N1), 30.17 (<u>C</u>–N3), 118.28 (<u>C</u>–N–naphthyl), 123.11, 123.42, 124.59, 125.05, 125.70, 129.34, 129.49, 135.98 (8<u>C</u>–naphthyl), 126.24 (<u>C</u>5–purine), 146.01 (<u>C</u>8–purine), 151.79 (<u>C</u>6–purine), 151.60 (<u>C</u>2–purine), 156.56 (<u>C</u>4–purine), 172.57 (<u>C</u>–OH–naphthyl). MS (*m/z*, %): 350 (M^+, 89). HRMS calc. for $C_{17}H_{14}N_6O_3$: 350.1127, Found: 350.1181.

2.4.2.4. 4-Amino-2-((1,3-dimethyl-2,6-dioxo-2,3,6,7-tetrahydro-1H-purin-8-yl)diazenyl)-3-methyl-6-oxo-6,7-dihydrothieno[2,3-b]pyridine-5-carbonitrile (11). Reddish brown crystals (4.4 g, 53 %). M.p. > 300°C. IR: ν_{max}= 3335 (NH_2), 3141 (NH), 2188 (CN), 1689 (CO), 1659 (CO), 1569 (C=N) and 1421 (CH_3) cm^{-1}. ^{1}H NMR: δ/ppm = 2.46 (s, 3H, C<u>H</u>$_3$–thienopyridine), 3.29 (s, 3H, C<u>H</u>$_3$–N1), 3.42 (s, 3H, C<u>H</u>$_3$–N3), 4.22 (s, 2H, N<u>H</u>$_2$), 10.70 (s, 1H, N<u>H</u>-pyridyl), 11.30 (s, 1H, N<u>H</u>–N7). ^{13}C NMR: δ/ppm = 6.49 (<u>C</u>H$_3$-thienopyridine), 27.89 (<u>C</u>-N1 purine), 30.24 (<u>C</u>–N3 purine), 81.42 (<u>C</u>5–thienopyridine), 112.69 (<u>C</u>2–thienopyridine), 113.21 (<u>C</u>9–thienopyridine), 117.56 (<u>C</u>≡N), 128.14 (<u>C</u>5–purine), 134.32 (<u>C</u>3–thienopyridine), 146.01 (<u>C</u>8–purine), 150.75 (<u>C</u>6–purine), 151.49 (<u>C</u>2–purine), 151.75 (<u>C</u>4–purine), 159.95 (<u>C</u>8–thienopyridine), 166.33 (<u>C</u>6–thienopyridine), 172.88 (<u>C</u>4–thienopyridine). MS (*m/z*, %): 411 (M^+, 34). HRMS calc. for $C_{16}H_{13}N_9O_3S$: 411.0862, Found: 411.0891.

2.4.3. Synthesis of (Z)-N-(1,3-dimethyl-2,6-dioxo-2,3,6,7-tetrahydro-1H-purin-8-yl)-2-(phenylamino)-2-thioxoacetohydrazonoyl cyanide (13)

2-Phenylthiocarbamoyl derivative **7** (4.4 g, 20 mmol) was suspended in 25 mL EtOH and 20% aq. KOH (30 mL). The mixture was kept in refrigerator overnight. A freshly cooled solution of diazonium chloride **3** (4.2 g, 20 mmol), which freshly prepared by adding cold sodium nitrite solution (1.38 g, 20 mmol) to cold suspension of **2** (3.9 g, 20 mmol) in 12 mL concentrated HCl, was then added dropwise with stirring to the cold suspension of **(7)**. The reaction mixture was allowed to stir at (0–5 °C) for additional 2 h and kept overnight in refrigerator. The solid azo product **(13)** was filtered off, dried and recrystallized from ethanol-DMF mixture as yellowish orange crystals (4.5 g, 59 %). M.p. > 300°C. IR: ν_{max}= 3204 (NH), 2202 (CN), 1644 (CO), 1597 (CO), 1539 (C=N) and 1442 (CH_3) cm^{-1}. ^{1}H NMR: δ/ppm= 3.14 (s, 3H, C<u>H</u>$_3$–N1), 3.33 (s, 3H, C<u>H</u>$_3$–N3), 7.24–7.44 (m, 5H, Ar–<u>H</u>), 8.64 (s, 1H, N<u>H</u>–purine), 9.77 (s, 1H, N<u>H</u>–Ph), 10.87 (s, 1H, C=N-N<u>H</u>). ^{13}C NMR: δ/ppm= 28.23 (<u>C</u>–N1 purine), 30.21 (<u>C</u>–N3 purine), 108.36 (<u>C</u>=N-NH), 118.71 (<u>C</u>N), 122.34 (<u>C</u>5–purine), 124.17, 124.94, 125.71, 128.96, 129.31, 139.98 (6<u>C</u>–phenyl), 144.01 (<u>C</u>8–purine), 149.56 (<u>C</u>4–purine), 151.56 (<u>C</u>2–purine), 153.08 (<u>C</u>6–purine), 180.15 (<u>C</u>=S). MS (*m/z*, %): 382 (M^+, 51). HRMS calc. for $C_{16}H_{14}N_8O_2S$: 382.0960, Found: 382.0953.

2.4.4. Synthesis of (Z)-N-(1,3-dimethyl-2,6-dioxo-2,3,6,7-tetrahydro-1H-purin-8-yl)-benzothiazole-2-carbohydrazonoyl cyanide (14)

A solution of purinyl azo *N*-phenylethanethioamide **13** (7.6 g, 20 mmol) in 20 mL of ethyl acetate was prepared and pyridine (1.6 mL) was added dropwise during 10 min with stirring. A bromine solution was prepared (0.16 g, 20 mmol) in 10 mL ethyl acetate and added to the mixture dropwise during another 10 min stirring. The mixture was stirred continuously for further 3 h. The product was collected and recrystallized from ethanol as faint brown crystals (5.5 g, 73 %). M.p. > 300°C. IR: ν_{max}= 3292 (NH), 2175 (CN), 1731 (CO), 1628 (CO), 1548 (C=N purine) and 1389 (CH_3) cm^{-1}. ^{1}H NMR: δ/ppm= 3.21 (s, 3H, C<u>H</u>$_3$–N1), 3.41 (s, 3H, C<u>H</u>$_3$–N3), 7.21–8.02 (m, 4H, Ar–<u>H</u>), 10.47 (s, 1H, C=N-N<u>H</u>), 11.13 (s, 1H, N<u>H</u>–purine). ^{13}C NMR: δ/ppm= 28.20 (<u>C</u>–N1 purine), 30.27 (<u>C</u>–N3 purine), 117.66 (<u>C</u>N), 119.95 (<u>C</u>5–purine), 116.14, 116.87, 126.27, 127.09, 132.47, 136.71 (<u>C</u>=N-NH), 150.91 (6<u>C</u>–benzothiazole), 141.51 (<u>C</u>8–purine), 149.33 (<u>C</u>4–purine), 152.07 (<u>C</u>2–purine), 154.48 (<u>C</u>6–purine), 159.95 ((<u>C</u>(S) benzothiazole)). MS (*m/z*, %): 380 (M^+, 18). HRMS calc. for $C_{16}H_{12}N_8O_2S$: 380.0804, Found: 380.0799.

2.4.5. General method for synthesis of 1,3-dimethyl-7substituted-1H-imidazo[2,1-f]purine-2,4(3H,8H)-dione derivatives (16a-c)

A mixture of equimolar quantities (20 mmol) of 8-amino-1,3-dimethyl-3,4,5,7-tetrahydro-1*H*-purine-2,6-dione **2** (3.9 g, 20 mmol) in warm DMF (50 mL) and α-halo carbonyl compounds **15a–c** (3.8 g phenacyl chloride, 2.2 g chloroacetyl chloride and 2.4 g ethyl chloroacetate, respectively) in presence of anhydrous potassium carbonate (4.2 g, 30 mmol) was refluxed for 10 h (monitored by TLC using ethyl acetate as eluent). The mixtures were then concentrated under vacuum and poured onto acidified ice-cold water. The collected precipitates were recrystallized from ethanol-DMF mixture.

2.4.5.1. 1,3-Dimethyl-7-phenyl-1H-imidazo[2,1-f]purine-2,4(3H,8H)-dione (16a). Greenish yellow crystals (4.6 g, 78 %). M.p. > 300°C (Lit. > 310 °C) [34].

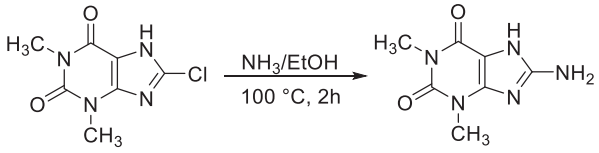
2.4.5.2. 1,3,7-Trimethyl-1H-imidazo[2,1-f]purine-2,4(3H,8H)-dione (16b). Light brown crystals (2.8 g, 60 %). M.p. > 300°C. IR: ν_{max}= 3150 (NH), 1688 (CO), 1539 (CO), 1489 (C=N purine) and 1406 (CH_3) cm^{-1}. ^{1}H NMR: δ/ppm= 1.86 (s, 3H, CH_3), 3.19 (s, 3H, CH_3–N1), 3.31 (s, 3H, CH_3–N3), 6.44 (s, 1H, CH=) and 11.28 (s, 1H, NH). ^{13}C NMR: δ/ppm= 13.92 (<u>C</u>H$_3$ imidazole), 28.23 (<u>C</u>–N1 purine), 30.26 (<u>C</u>–N3 purine), 100.84 (<u>C</u>5–purine), 104.26, 147.29 (2<u>C</u>–imidazole), 148.69 (<u>C</u>4–purine), 151.68 (<u>C</u>8–purine), 152.14 (<u>C</u>2–purine), 153.45 (<u>C</u>6–purine). MS (*m/z*, %): 233 (M^+, 18). HRMS calc. for $C_{10}H_{11}N_5O_2$: 233.0913, Found: 233.0902.

2.4.5.3. 1,3-Dimethyl-1H-imidazo[2,1-f]purine-2,4,7(3H,6H,8H)-trione (16c). Faint brown crystals (2.8 g, 54 %). M.p. > 300°C. IR: ν_{max}= 3292 (NH), 1628 (CO), 1588 (CO), 1548 (CO), 1478 (C=N purine), 1389 (CH_2) and 1349 (CH_3) cm^{-1}. ^{1}H NMR: δ/ppm= 3.32 (s, 6H, 2CH_3), 4.18 (s, 2H, CH_2) and 11.12 (s, 1H, NH). ^{13}C NMR: δ/ppm= 28.23 (<u>C</u>–N1 purine), 30.26 (<u>C</u>–N3 purine), 50.26 (<u>C</u>H$_2$, imidazole), 100.84 (<u>C</u>5–purine), 148.69 (<u>C</u>4–purine), 151.68 (<u>C</u>8–purine), 152.14 (<u>C</u>2–purine), 153.45 (<u>C</u>6–purine), 181.08 (C=O, imidazole). MS (*m/z*, %): 263 (M^+, 24). HRMS calc. for $C_9H_{19}N_5O_3$: 235.0718, Found: 235.0711.

3. Results and discussion

3.1. Chemistry

Successful preparation of 8-amino-substituted purine **(2)** by treatment of 8-chloro analogue **(1)** with ethanolic ammonia



1 2

Scheme 1. Synthesis of 8-amino-substituted purine **(2)**.

(Scheme 1) was very useful to produce prospective bioactive ingredients against malignant cell lines. The starting component was produced in high yield and in agreement with literature [32,33].

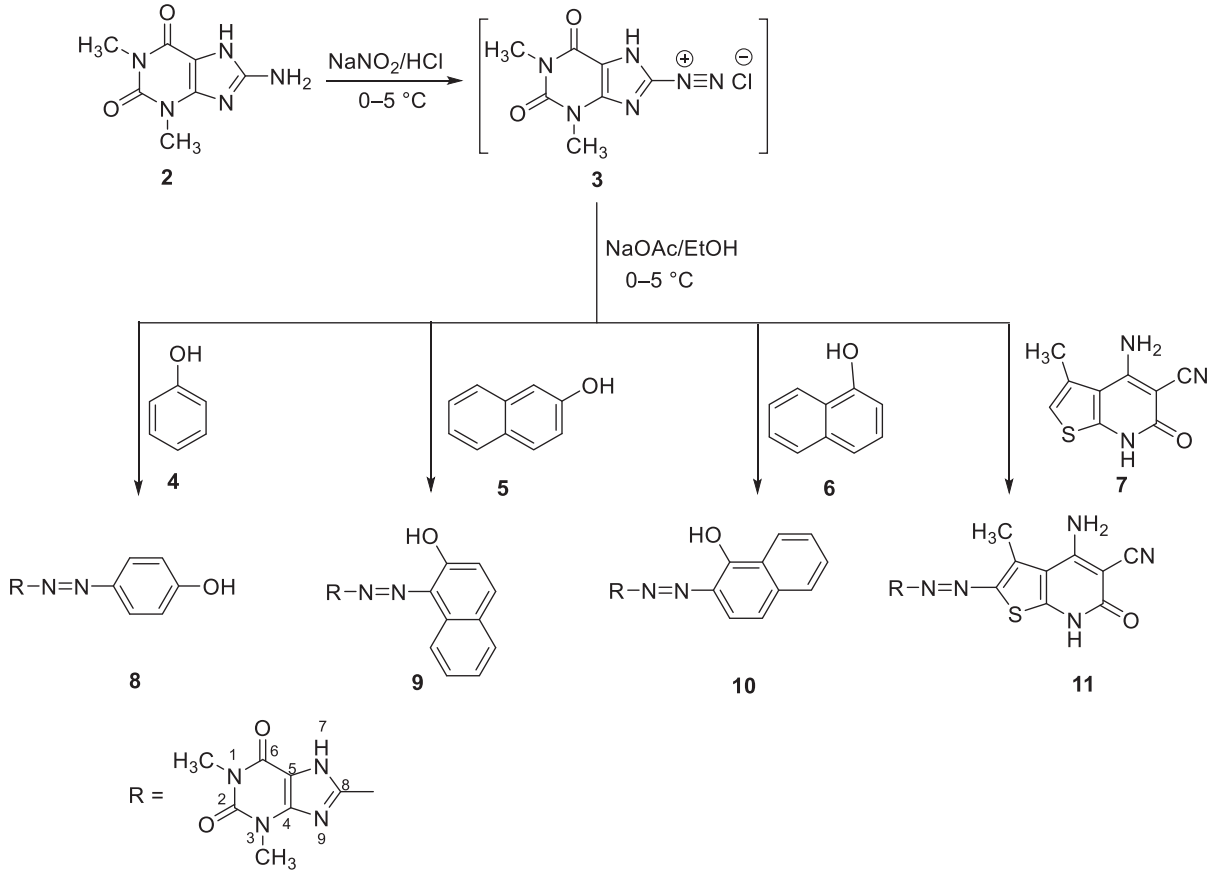
The target anticancer agents were planned to be synthesized through creating diazenyl derivatives of the starting 8-amino purine **(2)** and hence implementing imidazo and benzothiazolyl purines upon cyclization ability of active terminals. Diazotization of acidic solution of 8-amino purine **(2)** with sodium nitrite afforded the corresponding diazonium chloride **(3)** [32], which underwent coupling with aryl and/or hetaryl aromatic amines to afford the diazenyl derivatives **(8–11)** as described in Schemes 2 and 3. The products were structurally elucidated with various spectral analyses (c.f. experimental part).

Accomplishing different hydroxyl derivatives **(4–6)** was performed upon coupling with different hydroxyl couplers (i.e. phenol, α- and β- naphthols) yielding derivatives **(8–10)**, respectively, under cold condition (0–5 °C) and in presence of sodium acetate and ethanol (Scheme 2).

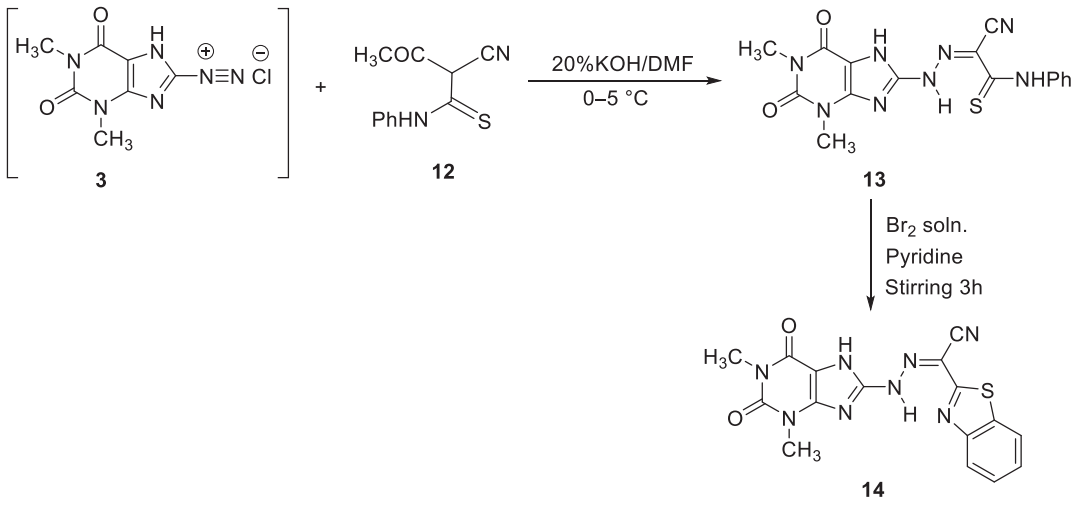
Inserting nitrile and amino functions could be achieved by coupling of diazonium salt **(3)** with thieno pyridine scaffold **(7)** to afford the diazenylthieno pyridine-5-carbonitrile compound **(11)**. The pyridine coupler **(7)** was exclusively pre-synthesized in our lab by the reaction of 2-aminothiophene derivative with ethyl cyanoacetate followed by ethoxide cyclization [35].

On the other hand, the 2-phenylthiocarbamoyl derivative **(12)** [36] was freshly prepared through the catalyzed base addition of phenyl isothiocyanate to cyanoacetamide as active coupler for Japp-Klingemann reaction. Therefore, coupling of 2-phenylthiocarbamoyl derivative **(12)** with appropriate diazonium salt **(3)** in potassium hydroxide proceeded in Japp-Klingemann reaction regime, causing amide cleavage [36] to afford the corresponding cyano-purine hydrazonoyl-*N*-phenylethanethioamide derivative **(13)** as indicated in Scheme 3. The IR spectrum of compound **(13)** showed disappearance of acetyl carbonyl at 1713 cm^{-1} and the singlet signal affiliated to methyl group at δ 2.2 ppm in the ^{1}H NMR spectrum. While cyano function at 2202 cm^{-1} was appeared. Extensively, cyclization of purin-2-thioxoacetohydrazonoyl cyanide compound **(13)** in bromine solution (Br_2 in ethyl acetate) in pyridine gave benzothiazolyl acetonitrile derivative **(14)** (Scheme 3). ^{1}H NMR spectrum displayed two signals (D_2O exchangeable) at δ 10.47 and 11.13 ppm due to two NH protons in addition to multiplet signal in the region of δ 7.21–8.02 ppm attributed to 4 aromatic protons.

Accordingly, 8-amino purine **(2)** underwent successful cyclization reaction on treatment with active α-halo carbonyl compounds **(15a-c)** (namely; phenacyl chloride, chloroacetyl chloride and ethyl chloroacetate) in presence of anhydrous potassium carbonate to yield the imidazo purine derivative **(16a-c)** as shown in Scheme 4. The structure is elucidated by the disappearance of characteristic signals of NH_2 (δ = 6.45 ppm) and purine NH (δ = 11.56 ppm) in the ^{1}HNMR spectrum, while presence of singlet signal at δ = 6.44 ppm and 11.12–11.28 ppm attributed to CH= and NH protons of imidazole ring, respectively.



Scheme 2. Synthesis of 8-substituted diazenyl-purine derivatives (**8**–**11**).

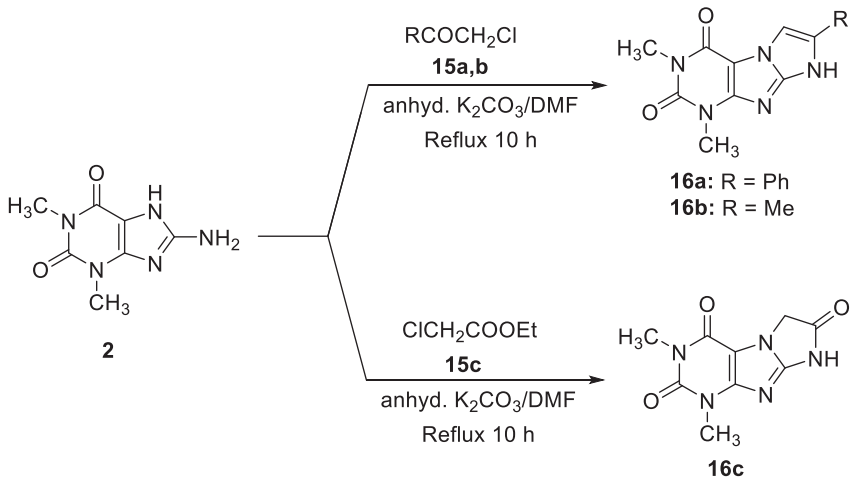


Scheme 3. Synthesis of cyano-purine diazenyl-*N*-phenylethanethioamide compound (**13**) and purine-diazenyl benzothiazolyl acetonitrile derivative (**14**).

3.2. Impact on the growth of human tumor cell lines

Evaluation of synthons *in vitro* for possible potential antitumor agents against different human cancers; Leukemia (HL60), Lung cancer (A549), Breast cancer (SKBR3) and Stomach cancer (MKN45), was performed after continuous exposure of 48 h. Cytotoxicity function has been determined by their cell growth inhibitory effect using the tetrazolium (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay [37]. IC_{50} values were then recorded as concentrations that inhibit 50% of tumor cell growth (Table 1). The more decrease of the IC_{50} value indicates higher efficiency against tumor cell.

Recorded data in Tale 1 indicates that derivatives **(9-11)** have high to moderate antitumor action in relatively low concentrations especially for Breast cancer cell lines (SKBR3). Although derivative **(9)** has high activity in low concentration (IC_{50}= 26.16 μM), but it's still not safe to normal cell lines along with compounds **(13, 14, 16b** and **16c;** IC_{50}= < 3- 33.15 μM**)**. Most of other tested compounds seem to be safe to normal cell lines in higher concentrations > 100 μM. These findings agree well with the molecular docking analysis.



Scheme 4. Synthesis of imidazo[2,1-*f*]purine-2,4(3*H*,8*H*)-dione derivatives (**16a-c**).

Table 1
Effect of the synthesized compounds **(8-11, 13, 14 and 16a-c)** on the growth of different tumor cell lines under investigation (*In vitro* Cytotoxicity IC_{50})

	***In vitro* Cytotoxicity IC_{50} (μM)**				
Cpd. No.	Leukemia HL60	Lung cancer A549	Breast cancer SKBR3	Stomach cancer MKN45	Lung normal WI38
8	> 100	87.48± 4.7	53.73± 3.8	58.07± 3.9	> 100
9	> 100	36.78± 3.1	26.16± 2.1	87.48± 4.7	< 3
10	> 100	64.54± 3.7	25.11± 1.9	62.61± 3.8	> 100
11	64.60± 3.8	80.22± 4.1	42.96± 3.2	79.62± 4.7	> 100
13	83.41± 3.4	95.17± 3.1	98.09± 2.7	96.48± 4.6	33.15± 1.2
14	88.12± 3.2	88.71± 4.3	92.70± 2.2	99.03± 2.5	23.41± 3.8
16a	> 100	99.25 ±5.1	52.21 ± 3.7	> 100	> 100
16b	83.05± 1.6	85.80± 4.1	93.51± 3.3	89.13± 3.1	18.60± 4.7
16c	78.21± 3.1	89.57± 3.2	77.19± 1.1	85.73± 4.5	32.14± 1.8

Means ± SEM of three-independent experiments

3.4. Structural activity relationship (SAR)

All compounds are capable to inhibit the growth of the tested human tumor cell lines with variable activities regarding to their chemical structure (Table 1). Inserting of hydroxyl phenyl feature showed superior tumor inhibition in lower concentrations especially for Breast cancer cell lines (e.g. Purine derivative **10**, IC_{50}= 25.11 μM). This phenomenon could be explained by its higher antioxidant and free radical scavenging action [38,39].

Table 2
Validation table energy score (kcal/mol) and rmsd

Compound	Energy score (kcal/mol)	rmsd
Reversine	-6.90	1.89
8	-5.32	1.41
9	-6.81	2.61
10	-6.54	0.849
11	-6.95	0.732
13	-7.01	1.60
14	6.99	1.80
16a	-6.21	1.47
16b	-5.84	1.00
16c	-5.66	0.90

3.5. Molecular docking

The study of molecular docks is an important technique to assess the potentially interactive relationship between the binding molecules and the target enzyme as well as receptor. On the basis of MOE 2015.10 device protocol [31], the validation of the docking of the synthesized purine derivatives **(8-11, 13, 14** and **16a-c)** and Reversine (reference molecule) was achieved [40]. A new class of Aurora inhibitors was identified as purine moeity [8-12] comparing drug validity between the synthesized purines and Reversine as a new category of pan-Aurora kinase blockers with strong emphasis viability against tumor in mice to propose a new platform for drug evolution. The validation of Aurora kinase in purine-derived complex based on measured ligand-protein interactive energies.

All the synthesized derivatives and reference molecule were virtually screened and analyzed. The selection of purine derivatives for potential inhibitors in the Aurora kinase pharmacopoeia was based on their effectiveness against SKBR3 cell lines. Docking the comparable molecules at the binding position of Aurora A led to a measured binding energy score of kcal/ mol (Table 2).

Reversine was used as a reference molecule and interaction with aurora kinase enzyme explained a very good drug ligand interaction (Fig. 1a,b) but gave bad drug ligand suitability, (rmsd= 1.89). This value was considered as a higher value compared with the synthesized purine molecules (Table 2).

It's of interest to discuss ligand interaction diagrams of the most active compounds **(10** and **11)** with Aurora kinase enzyme based on their experimental potency, while the rest diagrams will be included in Figures file (attached).

The dynamic 2D Aurora pocket with a purine derivative **(10)** describes how three electrostatic bonds interact (Fig. 2a). The 3D-crysyallophy structure explains the interaction between **(10)** as a ligand and Aurora pocket residues in which the hydrogen in the azepine ring attached with Leu99 and Lys180 (Fig. 2a).

The superposition of purine **(11)** generated a pharmacophore with H-bond with the active site of enzyme (Fig. 3a). Active site protein analysis was conducted from the same amino acid residue database, Leu223, Val107, Ala120, Lys101, Leu154, Leu170, Pro174,

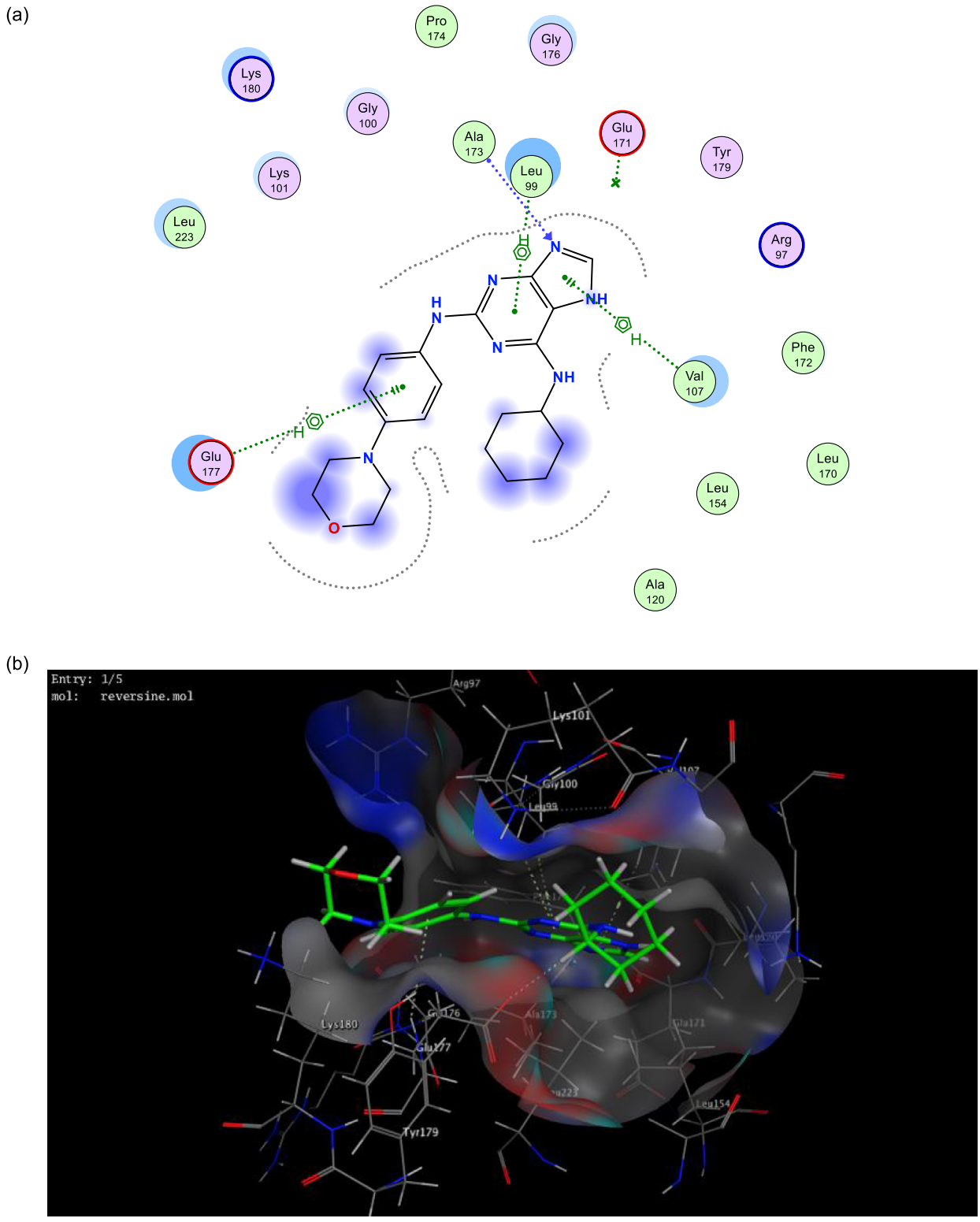
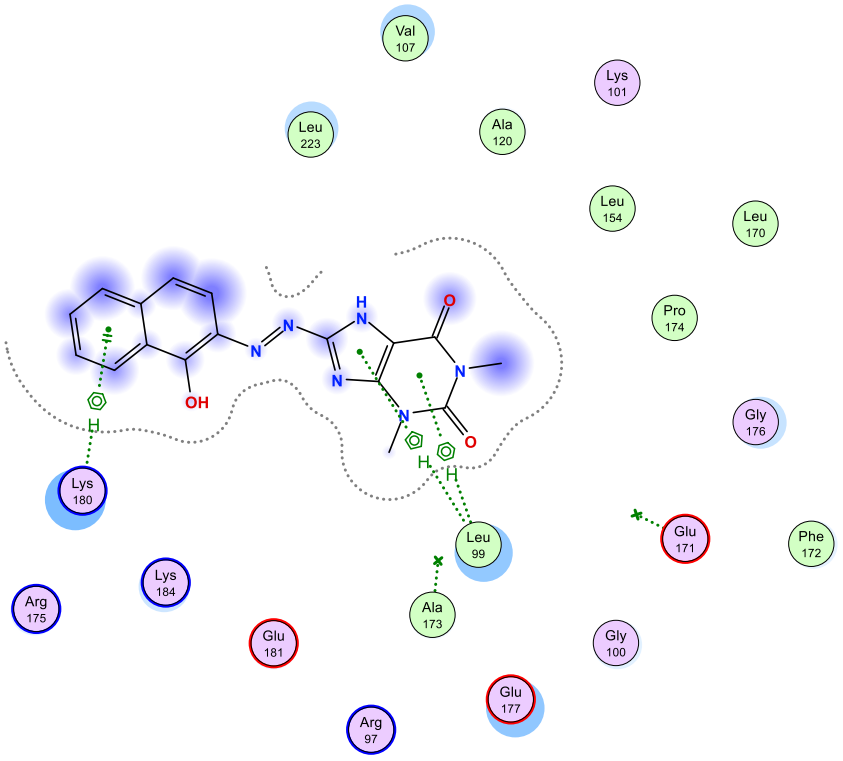


Fig. 1. **a** 2D interaction diagrams of Reversine (reference molecule) with Aurora kinase enzyme. **b** 3D interaction diagrams of Reversine with Aurora kinase enzyme.

(a)



(b)

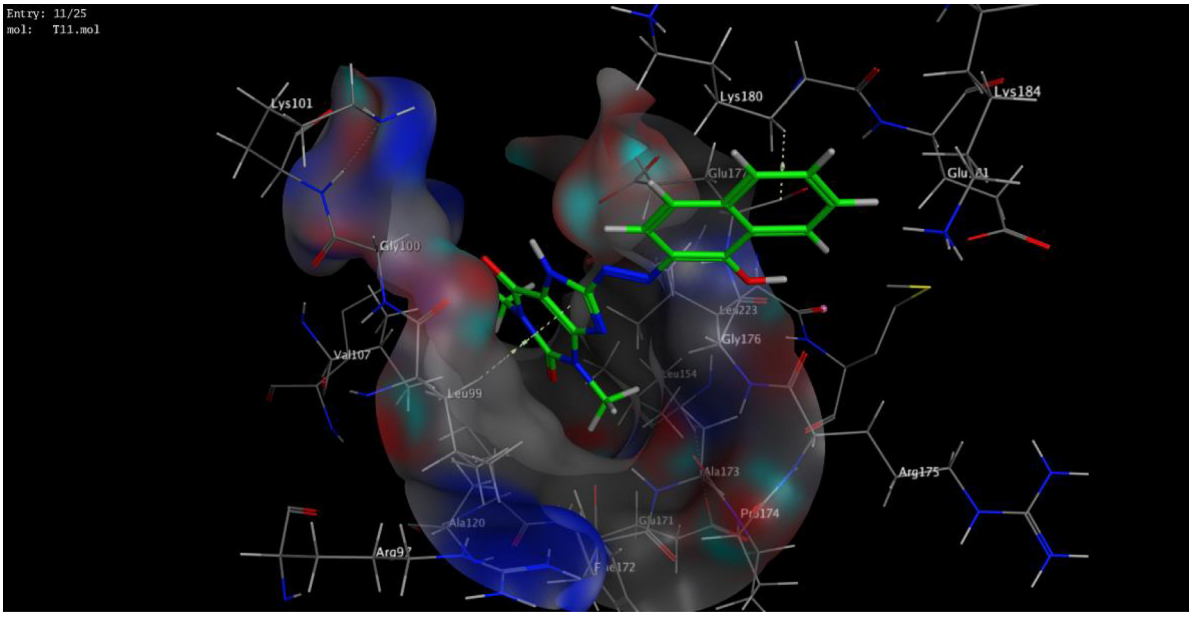


Fig. 2. **a** 2D interaction diagrams of compound **(10)** with Aurora kinase enzyme. **b** 3D interaction diagrams of compound **(10)** with Aurora kinase enzyme.

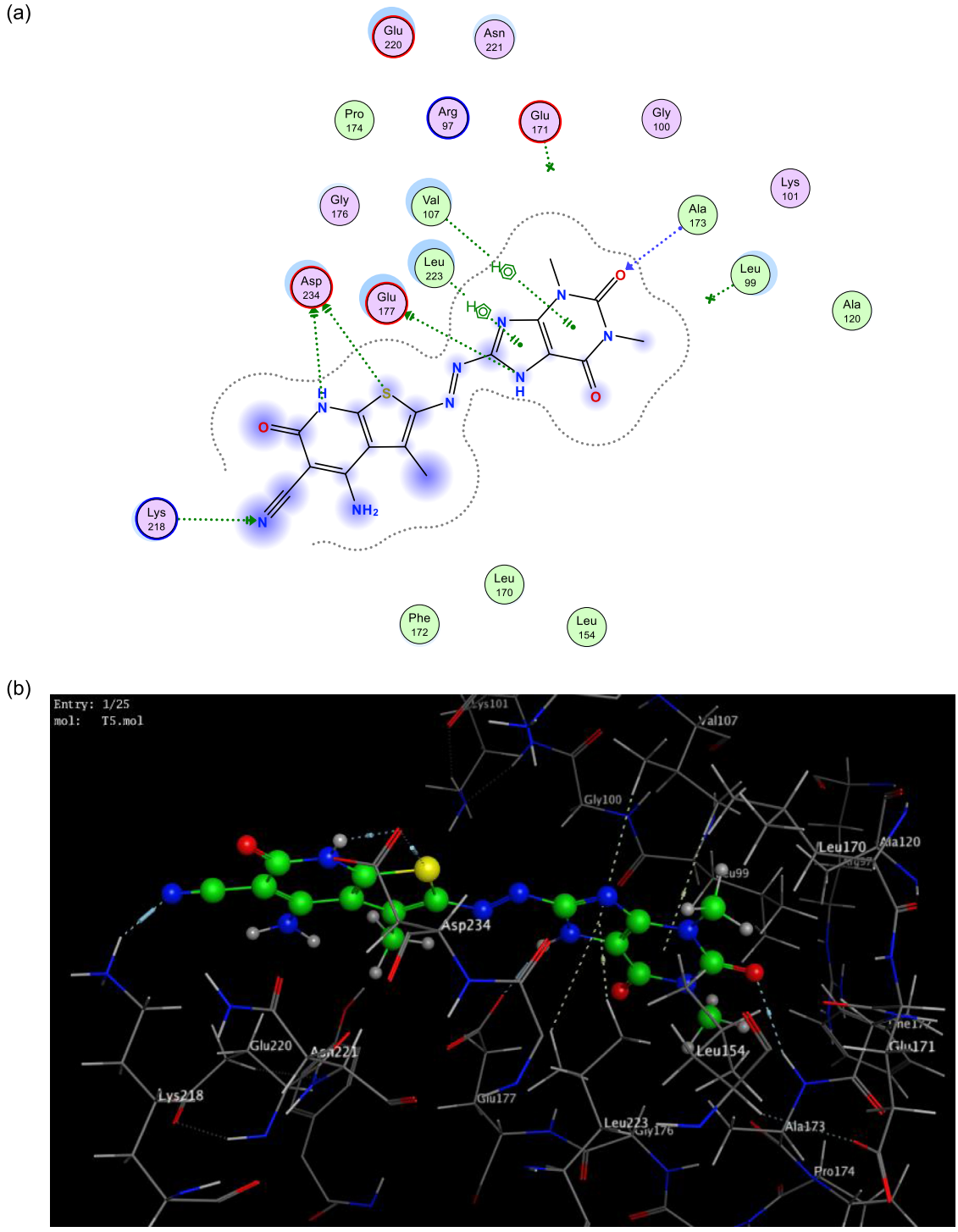


Fig. 3. **a** 2D interaction diagrams of compound **(11)** with Aurora kinase enzyme. **b** 3D interaction diagrams of compound **(11)** with Aurora kinase enzyme.

Gly176, Glu171, Phe172, Gly100, Leu99, Ala173, Arg97, Glu181, Lys184, Lys180, Arg175. The 2D pocket of Aurora kinase in a ligand complex with purine **(11)** explains that four hydrogen bonds and two π-π stacking. (Fig. 3b) Fig. 4.

3.5.1. The drug-ligand electrostatic force distance measurements

The drug-ligand electrostatic force distances represent the good drug-ligand interaction. For example, compound **(11)** presented 7 intramolecular forces indicating high drug ligand interaction (i.e. H- bond distances between lys218 and cyano group= 2.23; Asp234

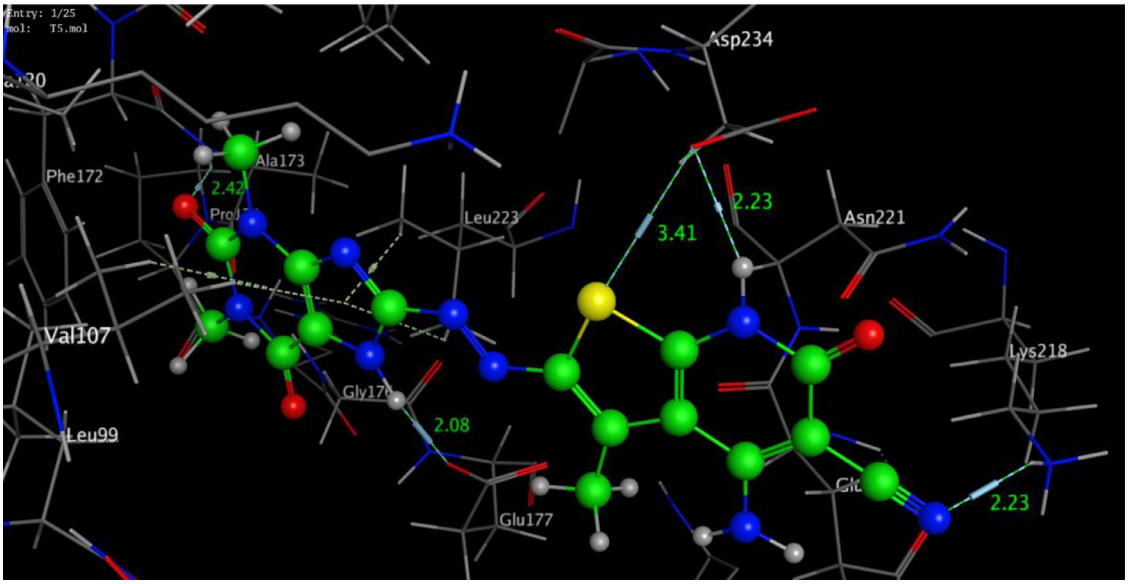


Fig. 4. The drug-ligand electrostatic force distance measurements for compound **(11)**.

and NH, S= 2.23, 3.41; Gly 177 and NH= 2.08 and Ala 173 and carbonyl group= 2.42). The π-π stacking also is existed between Val 107 and the two aromatic rings.

4. Conclusion

The newly synthesized purine-hybrids **(8-11, 13, 14** and **16a-c)** were *in-vitro* evaluated for their potent anti-cancer action against Leukemia (HL60), Lung cancer (A549), Breast cancer (SKBR3) and Stomach cancer (MKN45) cell lines. In general, most of synthons were exhibited more potency against Breast cancer (SKBR3) cell lines, while rest of them were of no serious action due to their higher (IC_{50}). The structural activity relationship (SAR) study for the synthesized hybrids regarding antitumor activities was discussed. Selected synthons, based on biological evaluation, were docked compared with anticancer target enzyme (Aurora kinase) to adequately understand the interaction of each molecule inside the active site of the (Aurora kinase) enzyme. The results explain that purine-imidazole hybrids **(10)** and **(11)** give promised values among group members.

Credit Author Statement

Mohamed E Khalifa: Conceptualization, Methodology, Software interpretation, Data curation, Visualization, Investigation, Writing Manuscript, Reviewing and Editing.

Declaration of Competing Interest

The author declares that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Supplementary materials

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